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A SIMPLE TELEOST KIDNEY IN THE GENUS CYCLOTHONE

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Introduction

Because it is the unit of structure and function in all vertebrate kidneys, the renal tubule contains the answer to many questions concerning the production of urine. Yet any theory which attempts to assign the various steps in this process to separate regions of the tubule must take into account the surprisingly wide degree of variation in tubule structure among the vertebrate groups. The relation of the presence or absence of the glomerulus to urine production and to the osmotic balance between the organism and different sorts of environment has been clearly outlined by H. W. Smith (1932) and others. The control of water content and salt concentration depends not only upon the action of the glomerulus, but also upon resorption and excretion by other tubule portions, such as the loop of Henle in mammals, and upon entirely extra-renal factors, such as the excretion of urea and salts by the gills of fishes.

The problem of further localizing kidney functions among the remaining segmental differentiations in the tubule proves to be more difficult. The fish kidney has been the subject of several cytological and histophysiological investigations contributing to this question, but confusion has resulted from the extreme diversity of kidney types in this group. Very few are found to contain all the segments of the typical vertebrate nephron, and Marshall (1934) has concluded that only the proximal convolute is common to all. There are as yet few clues to the benefits conferred by the other segments upon those fish which possess them. Among the forms which are able to adapt to sudden changes from fresh to salt water and back again, Grafflin (1937*a*) finds a lack of special development in any particular segment in relation to this remarkable ability, and concludes that it must be due to entirely extra-renal factors. Yet he does find indications of homologies in proximal convolute structure among those few which have been

studied cytologically (see below). It is obvious that there is a need for observations on more forms, especially because of the wide diversity of tubule types in the few already examined. The present study was undertaken with this fact in mind, and it was thought that some of the little known deep sea fish might present interesting modifications. The inaccessibility of their habitat, and the numerous strange adaptations of body structure which they have produced there, might be taken as indications of an early arrival in the sea, which would have given plenty of time for kidney structure to reach the aglomerular condition considered most logical for marine forms. Actually, the few genera so far sectioned all show glomeruli. One of these, however, shows a strikingly simple type of kidney, and forms the subject of this paper.

TABLE I

Data on the seven Cyclothone sectioned for study of kidney in situ

Series Number	Length in mm.	Depth taken	Fixation	Serial sections	Portion of kidney included
1	55	—	Formalin and sea water	10 μ	Complete
2	40	—	Modified Held's	10 μ	Complete
3	56	0–2000 m.	Bouin's	5 μ	Complete
4	28	600 m.	Bouin's	5 μ	Complete
5	26	600 m.	Bouin's	5 μ	Complete
6	40	—	Modified Held's	10 μ	Posterior, from proximal convolute 2nd part to end.
7	45	—	Formalin and sea water	10 μ	Anterior, as far as proximal convolute 2nd part.

MATERIAL AND METHODS

The fish used in this investigation is a small deep sea form belonging to the genus *Cyclothone*. It is long and very slender, without eyes, and supplied with numerous minute photophores. The specimens range from 26 to 56 mm. in length, with corresponding diameters of 3 to 6 mm. respectively. They were obtained in the vicinity of Bermuda, mostly in open-net hauls at depths of 0 to 2,000 m. Some were caught in closing-net hauls at 600 meters and other depths. The collections were made from the ketch Atlantis of the Woods Hole Oceanographic Institution during the summers of 1936 and 1937, and specimens for

the present work were obtained through the courtesy of Dr. J. H. Welsh of Harvard University.

The catch from each haul was usually preserved en masse in formalin and sea water. The extremely delicate body of the fish was shorn of much of the integument by crowding and rough treatment in the net, and was further twisted and distorted, and fixed in this state. Thus it was difficult to find a specimen in a straight condition suitable for sectioning. A few had been fixed separately in Bouin's or a modified Held's, but were in no better condition. None had been cut open, so that fixation was relatively poor. A group of those showing signs of better than average fixation plus a minimum of distortion was selected for a study of the kidney in situ by means of serial sections of the whole animal. In five of these, enough of the bodies were sectioned to include the entire urinary systems, which are reproduced approximately to scale (Figs. 1–5). A smaller portion of two others also was sectioned. All were stained with Harris hæmatoxylin and eosin. The data concerning the seven are given in Table I.

Observations

The *Cyclothone* kidney consists of a single pair of renal tubules extending parallel to the long axis of the body. They begin as two large, closely approximated Bowman's capsules, located dorsally at about the level of the last gill arches, and run a practically straight course side by side until they unite to form the bladder anterior to the anus. An investment of hemopoietic tissue surrounds the anterior half of the tubules, and just before entering the bladder they pass through a mass of glandular tissue which is probably the organ of Stannius.

Determination of the exact amount of regional differentiation in this nephron is difficult in the material available at present. There is a glomerulus, a neck segment, and a proximal convoluted segment of two portions. These four regions are marked off by histologically abrupt transitions. The second portion of the proximal convolute is followed by a thin intermediate segment, and this by a terminal segment somewhat like the distal convolute described in many tubules. These last two, however, are not set off by abrupt shifts in cell types. It is necessary to describe a long transitional region before the intermediate segment and, although the terminal segment appears rather suddenly, there is not a sharp histological transition from the intermediate segment.

Glomerulus and Neck

The capsule of Bowman is large, with a well-vascularized glomerular tuft which lacks the central avascular core observed in bird and reptile

nephron by Vilter (1935). There is a large amount of intracapsular space, but it is not filled with coagulum, and does not resemble the degenerative enlargements or outpouchings seen by Grafflin (1929) in the goosefish capsule.

The two capsules are separated by a thin septum (Fig. 6) which becomes indistinct as it passes between the capillary tufts, so that these latter structures appear to form a single unit. It was not determined whether there was a separate blood supply for each side of the tuft.

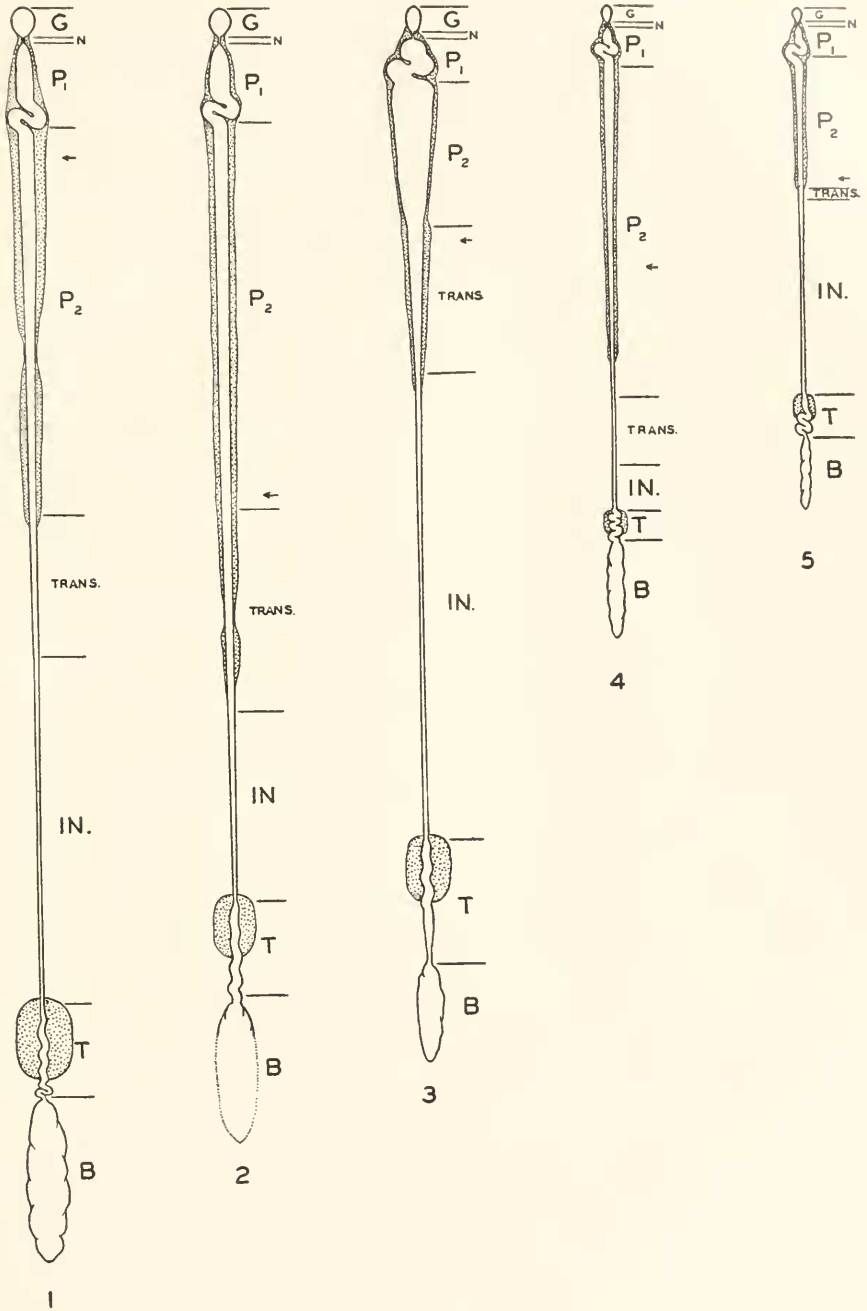
The short neck segments of the two tubules arise ventrolaterally from the glomerular capsules, and there is an almost immediate transition to the cells of the proximal convolute (Fig. 6). The neck cells bear cilia, but these are relatively few and very fine, requiring oil immersion for identification. The segment therefore does not give a picture comparable to the ciliated neck of other nephrons, such as that described by Guyton (1935) in the lungfish. The neck cells are of a low cuboidal type, 5 to 8 μ in height. Nuclei are small, irregular in shape, and stain densely. The cytoplasm also takes a hæmatoxylin stain. This is a short segment in all specimens, usually less than 100 μ. The diameter is small, but the lumen relatively large, so that there seems no question as to the functional nature of the glomerulus.

First Portion of Proximal Convolute

The cells of this region appear abruptly (Fig. 6). In the first few sections, the tubule wall shows a peculiar tufted structure due to the fact that the cell height varies from 12 to 50 μ (Figs. 7 and 8). Very soon, however, the cell height becomes comparatively constant (Fig. 9). Two of the specimens fail to show this irregularity.

In one member of the series (No. 7) the cells of the "tufted" region just described are filled with very large granules (Fig. 10) which crowd the nuclei against the basement membrane. These granules are spherical in shape and appear homogeneous in composition. Most of them stain a brilliant red with eosin, although a few remain colorless. Other specimens show the granules to a lesser extent, always smaller and fewer in number.

FIGS. 1 to 5. Diagrammatic reconstruction of kidneys of five *Cyclothone* specimens. × 10. Only one tubule is shown; the other joins it at beginning of bladder. Upper stippled areas, hemopoietic tissue; lower stippled areas, organ of Stannius. *G*, glomerulus; *N*, neck segment; P_1 first portion of proximal convolute; P_2, second portion of proximal convolute; *TRANS.*, transitional zone; *IN.*, intermediate segment; *T*, terminal segment; *B*, bladder. Arrows indicate level of appearance of first ciliated cells. Numbers correspond to those of the series described in Table I.


G
N
P1
P2
TRANS.
IN.
T
B
1
G
N
P1
P2
TRANS.
IN
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B
2
G
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P1
P2
TRANS.
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T
B
3
G
N
P1
P2
TRANS.
IN.
T
B
4
G
N
P1
P2
TRANS.
IN.
T
B
5

FIGS. 1–5

The apical cytoplasm is always more or less granular, while that below the nucleus shows prominent striations, with fibrillæ arranged parallel to the long axis of the cell and closely packed together. The cytoplasm takes a deep eosin stain. There is a uniform brush border, rather coarsely fibrillar, and 5 to 8 μ in height.

Nuclei are oval, 4 to 5 μ in diameter, and heavily stained. Their position within the cell varies widely. In some specimens, they are located below the center of the cell in the first few sections, but later become central or even apical. In one series the nuclei are in the basal half of the cell throughout, while in another the position is always in the apical half (Figs. 7 and 8). The tubule enlarges greatly toward the end of this region, and convolutions appear. These always have the same simple structure, a double fold (Figs. 1 to 5).

Second Portion of Proximal Convolute

At this point there is an abrupt change (Figs. 11 and 12) to a cell type with lighter staining cytoplasm, larger nucleus, and high, irregular brush border. The cytoplasm shows very few granules, but is still striated, with fibrillæ loosely packed so that the cell appears less dense. The brush border becomes exceedingly irregular in form (Figs. 12 and 13) sometimes extending out into the lumen for a distance of 25 μ. Its structure is finely granular and sometimes vacuolated, without striations. Some portions have a droplet-like appearance as if about to be pinched off into the lumen, which suggests that these cells are engaged in some sort of apocrine secretion. In later sections, the border becomes lower and more regular, sometimes regaining a striated appearance.

The nuclei are larger than in the first portion and stain less heavily (Figs. 11 and 12). They are usually just above the center of the cell.

Fig. 6. Section of kidney at level of glomeruli, showing the close approximation of these bodies. Right tubule (above) sectioned at level of transition from neck (right) to P_1 (left). Other tubule (below) shows scattered P_1 cells among those of neck. Passage from glomerulus into neck is in an earlier section. × 180.

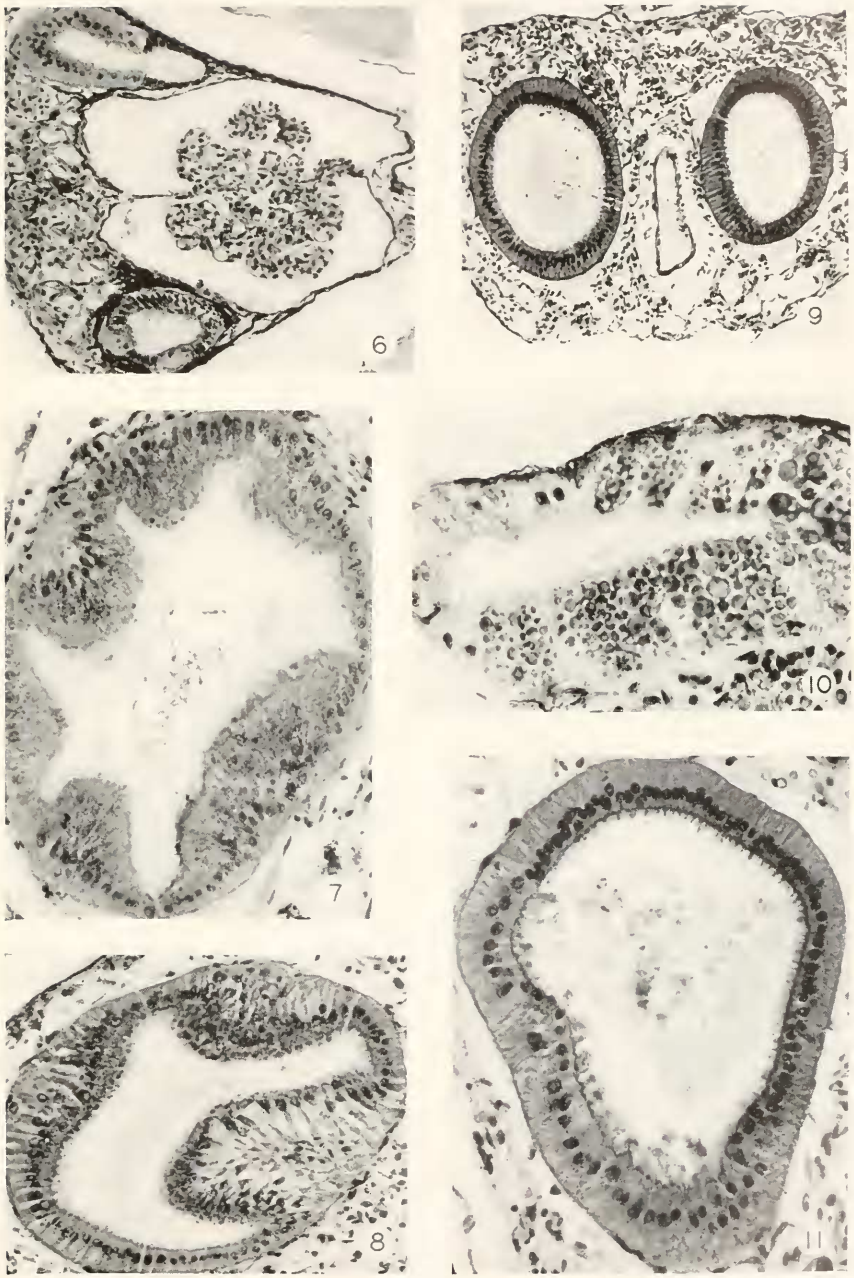
Fig. 7. Tufted region in early part of P_1; in this specimen the nuclei are basal and there is a large quantity of granular cytoplasm between them and the lumen. × 255.

Fig. 8. Same region of another specimen. Here the nuclei are apical, and there is much less of the granular cytoplasm. × 240.

Fig. 9. Central part of P_1. The tubule wall is even and the brush border regular. × 130.

Fig. 10. Cells of P_1, showing remarkable number of large granules. These occur in only one specimen; see text. × 535.

Fig. 11. Section passing through region of transition from P_1 (cells of upper right half) to P_2 (lower left). × 280.



FIGS. 6–11

Frequent double nuclei are seen, oriented in a line parallel to the vertical axis of the cell (Fig. 13).

The cells are wider than in the first portion, but only slightly higher, ranging from 15 to 20 μ in height near the transition from the first half of the segment. There is a steady decline in height of cell and brush border both, throughout this region.

Intermediate Segment

When the brush border is entirely gone and the cells are reduced to a cuboidal shape without prominent striations in the cytoplasm, a typical intermediate segment has been reached. This is separated from the proximal convolute by a long transitional zone in place of the abrupt change noted between the other segments. Two factors make it impossible to eliminate this zone. In the first place, the changes in cell height and nature of brush border take place gradually. The nucleus also slowly becomes smaller and more densely stained. In the second place, the cells on the medial side of each tubule retain the tall brush border, greater cell height, and fibrillar cytoplasm long after the cells of the rest of the epithelium are of the low cuboidal type of the intermediate segment, without brush border or cytoplasmic striations.

The tubule in the typical intermediate segment is very small and lies on the dorsal wall of the body cavity without any investment of hemopoietic tissue. It is usually less than 50 μ in diameter, and the cells are often much wider than high. The cytoplasm is homogeneous, dense, and rather heavily stained with eosin. Although the cell border is typically plain, there are frequently various sorts of bleb-like protrusions on the cell surfaces which are very much like a brush border.

FIG. 12. Same region under higher magnification. Note abruptness of transition; P_2 cells are on right. × 630.

FIG. 13. Typical high brush border of P_2. It is highest on the medial wall of each tubule. × 240.

FIG. 14. Whole section; the kidney tubules lie just above the oesophagus (center). Dorsal side uppermost. The heart may be seen ventrally. × 9.

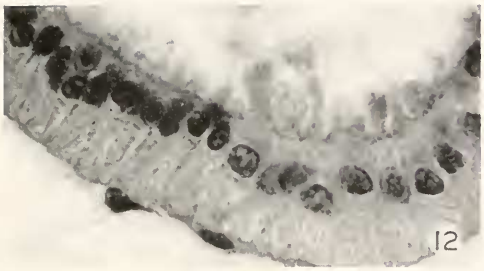
FIG. 15. Beginning of transitional zone. The epithelium is low, brush border is beginning to disappear (upper left) and ciliated cells are common. × 365.

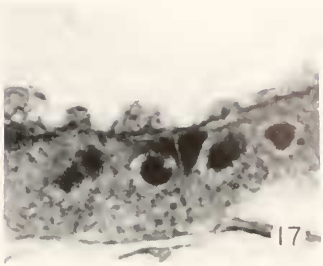
FIG. 16. Intermediate segment. Very low epithelium, no brush border, cilia numerous. Note small size of tubule. × 720.

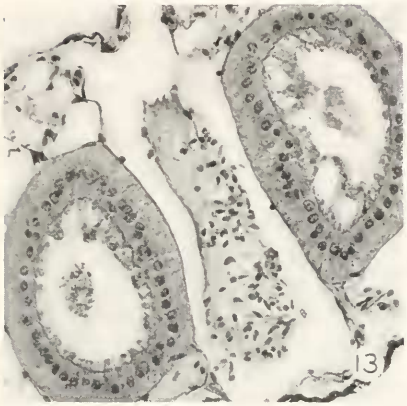
FIG. 17. Portion of tubule wall (P_2) to show a typical isolated ciliated cell. × 630.

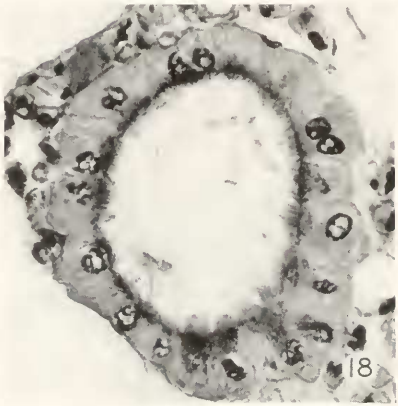
FIG. 18. Terminal segment. The strong suggestion of a brush border shown here is absent on most of the other specimens. × 645.

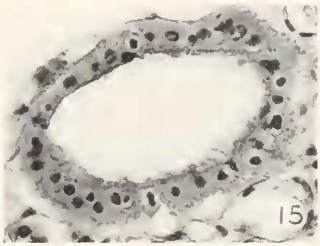
FIG. 19. Stannius organ, with kidney tubule at either end of glandular mass. × 90.

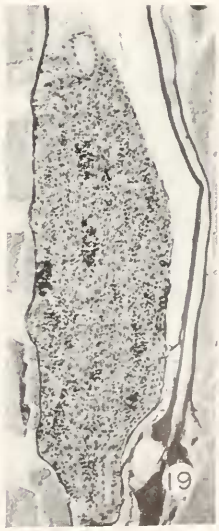












FIGS. 12–19

Cilia are fairly numerous, but are so extremely fine in texture that they never form a conspicuous feature of the tubule topography, and frequently require high power to be seen at all. Isolated ciliated cells first occur early in the second portion of the proximal convolute (Figs. 1 to 5). They are conspicuous as small cells without a brush border and with heavily stained nuclei, wedged between the other cells of the tubule wall (Fig. 17). The several cilia from each such cell are fused into one strand, only occasionally showing the individual elements. Rare at first, these cells become progressively more numerous up to the end of the intermediate segment, where there may be several in one cross-section (Fig. 15). In both structure and distribution they resemble the isolated ciliated cells in the second portion of the proximal convolute of the lungfish (Guyton, 1935).

Terminal Segment

As the tubule enters the organ of Stannius (Fig. 19) the cilia disappear, and there is a certain amount of restoration of the glandular type of epithelium. It shows an increase in thickness in all cases, usually about 20 per cent, but in two specimens the cells are doubled in height, and regain the striated cytoplasm. In one of these there is also a marked accumulation of basophilic granules in the apical region of the cell (Fig. 18). The cell margins are uneven and sometimes the roughness suggests a brush border (Fig. 18). In certain regions, however, the luminal border is smooth, while some of the nephrons show no sign of this surface modification at all, so that it is assumed this is not a brush border segment.

The transition to the terminal segment takes place within the distance covered by five or six sections, in one specimen, yet there is always a gradation of one cell type into the other, without an abrupt change.

It seems safe to conclude that there is no collecting segment, for the glandular epithelium frequently lasts unchanged to the point of union of the tubules at the bladder. Some tubules show a gradual return toward the structure of the intermediate segment, but the cells always remain somewhat taller.

Convolutions appear just before the start of the bladder. They are much less regular than the first set described above. The large specimens, which are females, show only a slight bending of the tubule, while in the small ones (Figs. 4 and 5) it may go through several tortuous loops. It is interesting to note that these smaller individuals are males, a fact which suggests that the extra convolutions might bear some relation to the "sexual segment" observed in certain vertebrate

male kidney tubules. However, no connection was observed between the testis and the kidney in either of the two males sectioned.

In the midst of these convolutions, the tubule leaves the organ of Stannius and descends ventrally to the bladder. This organ is lined with a low cuboidal epithelium, in which the curved tops of the cells give a characteristic biscuit-like appearance. A very thin coat of smooth muscle completes the structure. An external opening is found behind that of the digestive tube, and close to that of the reproductive tract, at least in the case of the female. Neither of the male specimens shows an open duct leading from the testis, although a very thin strand of tissue can be traced back from this organ toward the region of the anus.

Discussion

All the *Cyclothone* kidneys so far sectioned show a high degree of similarity between corresponding parts of the tubules from the glomerulus on through most of the second portion of the proximal convolute. Beyond this region differences are encountered in the nature of the epithelium, making it difficult to find characters common to all, and next to impossible to draw comparisons with other tubule types.

The confusion with regard to structure in this region is not entirely due to an unusual tendency toward individual variations. The proximal half of the tubule shows decidedly better fixation of important cellular features, probably because penetration was much faster there in the region of the gills than it was posteriorly where the kidney is surrounded by the thick muscle layers of the body wall (Fig. 19).

Audigé (1910) states that the typical teleost kidney may consist of three parts, the head, middle, and hind kidney. The head kidney in young fish contains two large glomeruli derived from the pronephros. These are rarely seen in adults, although Price (1910) describes one in *Bdellostoma stouti*. In *Cyclothone* there is a lack of any opening into the cœlom such as would be expected with a pronephric tubule, and in addition the glomerulus is not external. Furthermore, there is a direct passage toward a urinary bladder along a tubule whose epithelium is characteristic of the renal tubules of typical vertebrate mesonephroi. Nevertheless, the extreme anterior location of these two bodies suggests that they may be modified pronephric glomeruli, associated posteriorly with a pronephric duct which has undergone modification into a typical secretory tubule.

There is a remarkable variation in tubule sizes among the seven individuals sectioned. In the typical vertebrate kidney all tubules are identical, but their number varies with the size of the kidney and of the

animal containing it. Nash (1931) has pointed out the constancy of the ratio between tubule number and body surface in several fish. In the present example, however, the tubule number is constant. The amount of kidney tissue is nevertheless adjusted to the size of the animal, simply by a change in size and length of the tubules themselves. The size of the fish from which these tubules are taken is given in Table I.

Even the smallest of the five illustrated here is a rather large tubule in comparison with those of other fish. Table II lists the approximate measurements of the largest and smallest tubules of the *Cyclothone* series (Figs. 1 and 5). These may be compared with tubule sizes reported by Nash (1931) for a large number of species. Tubule lengths he found usually between 1 and 9 mm.; diameters ranged upwards from 25 μ. Glomeruli were commonly between 35 and 95 μ. But

TABLE II

Approximate measures of largest and smallest tubules of Cyclothone series

	Segment diameters (μ)				Total length
	Glomerulus	Prox. conv.	Interm.	Terminal	
Largest (No. 1)	250	200	50	60	14.2 mm.
Smallest (No. 5)	130	100	30	50	5.6 mm.

isolated examples in his list far exceed this range: the hagfish glomerulus is 500 μ in diameter, and the nephron of the skate attains a length of 19 mm. Therefore the *Cyclothone* nephron comes well within the limits observed by Nash. In only one specimen is the tubule diameter really exceptional; the proximal convolute of No. 3 measures 500 μ in several places.

The lack of special cytological preparations makes it difficult to homologize the finer structure of the different tubule regions with those of other kidneys. In his description of the cell types in the two portions of the proximal convolute of the sculpin, Grafflin (1937*c*) lists, among others, these three points of difference: the second part shows smaller nuclei, a more eosinophilic cytoplasm, and lower brush border, than the first. This is exactly the reverse of the condition as regards these three characters in the *Cyclothone* proximal convolute divisions. This suggests that the two portions of the proximal convolute in these two animals should be homologized in reverse order. Yet throughout the whole proximal convolute of the *Cyclothone* nephron, the cells show

a greater density of the cytoplasm on the luminal side of the nucleus, which is just the opposite of the condition reported in the sculpin.

In attempting to set up a general picture of homologies among the different fish kidneys, Grafflin (1937*d*) concludes that the cell type of the second half of the proximal convolute of the sculpin is homologous with the same region in the tubule of the eel, and with the entire proximal convolute of the toadfish which shows no signs of division into two parts. Also, the first portion of the proximal convolute in eel and sculpin presents a second homology. Returning to *Cyclothone,* if we try to make a comparison between the first part of the proximal convolute, and the second part of that segment in the eel, as we tried to do with the sculpin above, we find again the same similarity as to eosinophilic cytoplasm and lower brush border. The difference in nuclear size, however, is not mentioned in Grafflin's description of the eel nephron (1937*b*), and our comparison is further weakened by the appearance of his figure showing the point of transition between the two cell types. This photograph shows a much larger cell in the second portion of the tubule and suggests that the homology with *Cyclothone* may well be a direct one, part one with part one and part two with part two. This view is further strengthened by the fact that a "zone of granular cells" sometimes appears in the first half of the proximal convolute of the eel, just as does a similar zone in one of the *Cyclothone* specimens (see above and Fig. 10).

The nephron of the lungfish, *Lepidosiren paradoxa,* according to the description of Guyton (1935) is quite similar in size to that of the larger cyclothones, and except for having an initial collecting segment, it is divided into the same general regions. Grafflin finds that this tubule does not fit very well into his scheme of homologies among proximal convolutes. The first portion of that segment in the lungfish resembles the second in sculpin and eel, while the second part doesn't resemble anything at all in the other two. The principal cell in this second part has an inconspicuous brush border and lacks prominent vertical striations in the cytoplasm. While these characters make it as foreign to *Cyclothone* as to the eel and sculpin, it is nevertheless worth noting that it shows a denser apical cytoplasm much like the cells of the proximal convolute in the *Cyclothone* nephron. The shape and distribution (increasing toward the distal end) of the ciliated cells in the second part of that segment is similar in these two fish also.

It should be emphasized that the problem of homologies between cell types of the various segments in different tubules depends upon the constancy of the characters chosen for comparison. In the sculpin, the nuclei of the cells of the first part of the proximal convolute are

described as being usually in a central position, although sometimes at the base of the cell. In the same region of the eel, nuclear position is stated as almost invariably basal. In the *Cyclothone* tubules, it is possible to compare areas at exactly the same distance along each segment. When this is done one finds, using the first part of the proximal convolute as an example, a variation all the way from basal nuclei to apical ones (Figs. 7 and 8). The coarsely granular cytoplasm in the apical region of cells in both these tubules suggests that this area is engaged in active secretion, and that the nuclear position may simply vary with changes in the cytoplasmic volume at the apical end. That such changes may follow a definite cycle can be seen from the histophysiological studies made by von Möllendorff (1937) on fish kidneys. He observes a secretory cycle involving changes in both cell border and cytoplasmic content. Such a series of repeated changes make it risky to consider many cytological features specific for one tubule region, until it be determined whether they are subject to change under various physiological states of the segment in question.

In conclusion I wish to state my sincere appreciation for the kindness of Professor A. B. Dawson of Harvard University in suggesting this problem and in offering valuable advice at all times in the course of the work.

Summary

The *Cyclothone* kidney is extremely simple. The entire system consists of two tubules running a practically straight course side by side and uniting at the bladder. They are surrounded anteriorly by hemopoietic tissue and posteriorly by the organ of Stannius.

The tubules can be divided into the following histologically distinct portions: glomerular capsule; neck segment; proximal convolute, with two types of brush border epithelium; ciliated intermediate segment; and terminal segment. An abrupt change in cell type separates the neck segment and the two parts of the proximal convolute, but the intermediate segment arises after a long zone of slow transition, and the cells of the terminal segment also appear gradually.

The cell types of the different regions of the nephron are described, with a discussion of the problems of homologies between tubule segments of different kidneys.

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